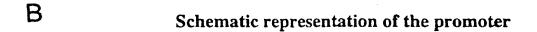


Α

## The HER-2/neu promoter



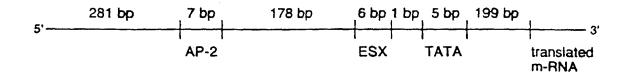
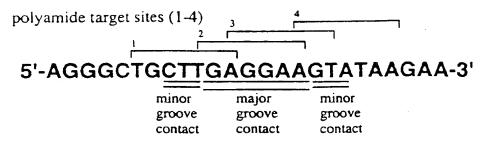


Fig. 1

PCT/US99/20489

## The ESX promoter site



B site 1 site 3 5'-T G C T T G A-3' 5'-A G G A A G T-3' 3'-T C C T T C A-5' 5'-A G G A A G T-3' 5'-T G C T T G A-3' site 2 site 4 5'-T G A G G A A-3' 5'-A G T A T A A-3' 5'-T G A G G A A-3' 5'-A G T A T A A-3'

Fig. 2

WO 00/15773 PCT/US99/20489

a) CF3COOH. b) A, (iPr2)NEt, DMF. c) Boc-β-alanine, HBTU, (iPr2)NEt, DMF. d) N-α-Fmoc-N-γ-Boc-D-diamino butyric acid, HBTU, (iPr2)NEt, DMF. e) C, HBTU, (iPr2)NEt, DMF. f) B, HBTU, (iPr2)NEt, DMF. g) 20% piperidine, DMF. h) (N,N-dimethylamino)propylamine; prep. HPLC.

Fig. 3

Fig. 4

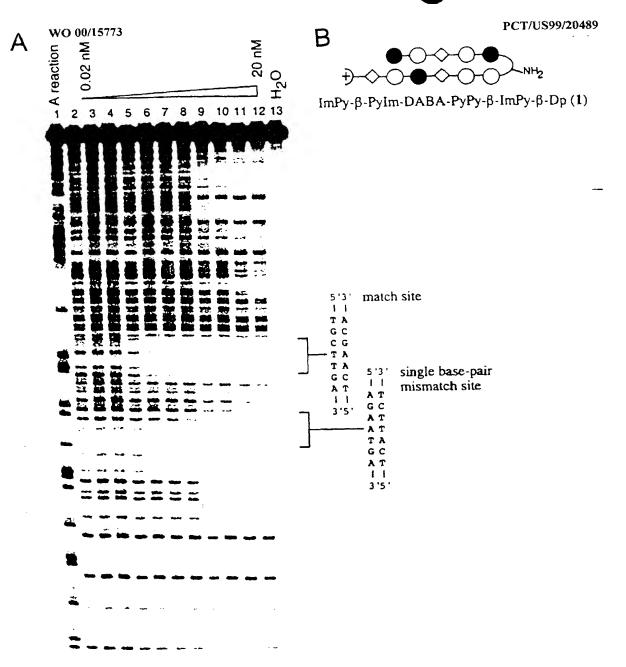


Fig. 5

PCT/US99/20489

Α 5'-AGGGCTGCTTGAGGAAGTATAAGAATGAAGTTGT-3'

B 5'-T G C T T G A-3'

TGCTTGA:  $K_a = 1.8 \cdot 10^{10} \text{ M}^{-1}$  (match site) AGAATGA:  $K_a = 6.1 \cdot 10^9 \text{ M}^{-1}$  (mismatch site)

(Binding of the Polyamide to this mismatch site may also lead to a decreased binding of the ETS protein.)

5'-T G A G G A A-3'

3'-A C T C C T

site 2

5'-T G A G G A A-3'

No binding observed at [Polyamide] ≤ 10 nM.

5'-T G C T T G A-3' 3'-A C G A A C T-5'

(at cal 5 nM).

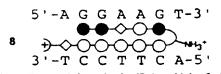
site 3

Weak binding to the match and the mismatch sites No binding to the match site for {Polyamide} ≤ 10 nM. At [Polyamide] = 1 to 10 nM: several footprints observed

5'-A G G A A G T-3'

3'-T C C T T C A-5' No binding to the match site for [Polyamide] ≤ 10 nM Single base-pair mismatch site (ATGAAGT):  $K_{\bullet} = 1 \cdot 10^9 \,\mathrm{M}^{-1}$ 

site 4 AGTATAA:  $K_2 = 1.5 \cdot 10^{10} \text{ M}^{-1}$  (match site)



Match site: weak footprint for [Polyamide] = 5 nM. Not very selective.

Single base-pair mismatch site (ATGAAGT):  $K_{\bullet} = 1 \cdot 10^9 \,\mathrm{M}^{-1}$ 

5'-A G T A T A A-3' AGTATAA:  $K_a = 9.5 \cdot 10^9 \text{ M}^{-1}$  (match site)

Fig. 6

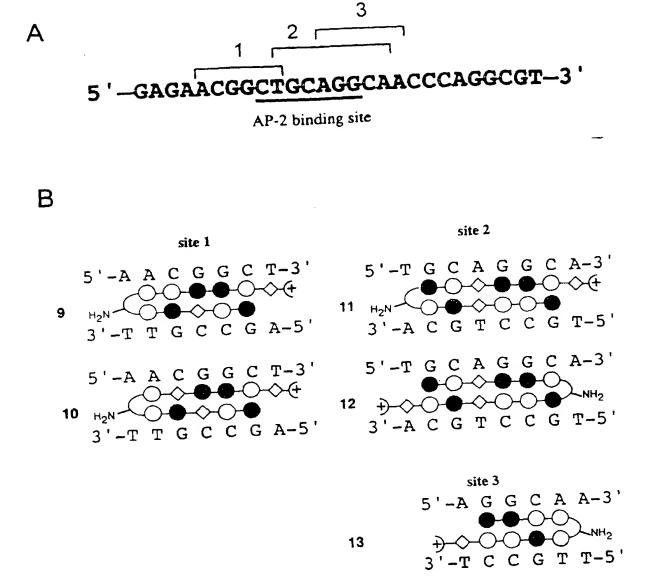


Fig. 7

Fig. 8

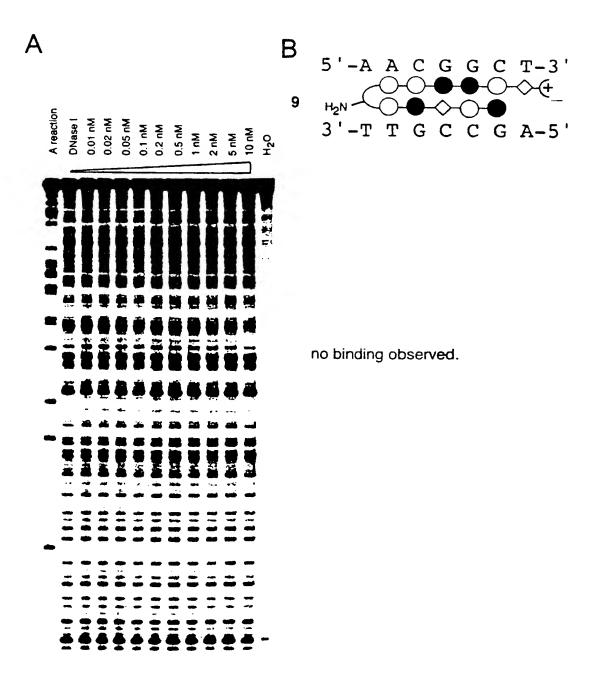


Fig. 9

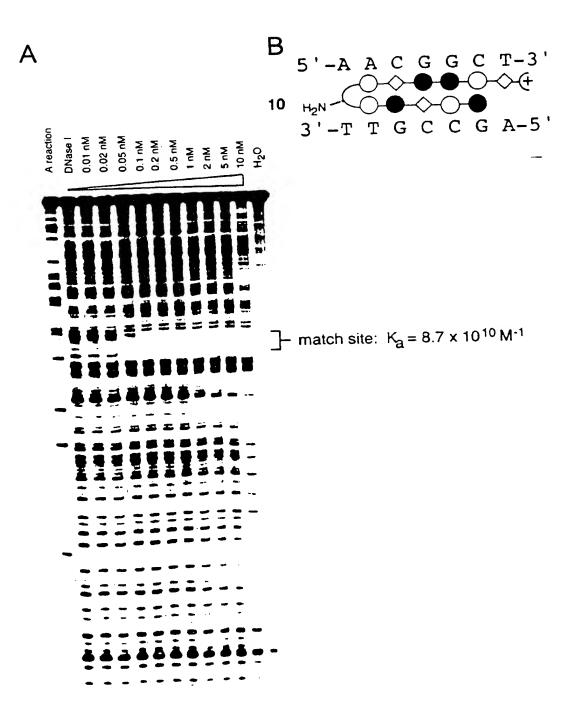


Fig. 10

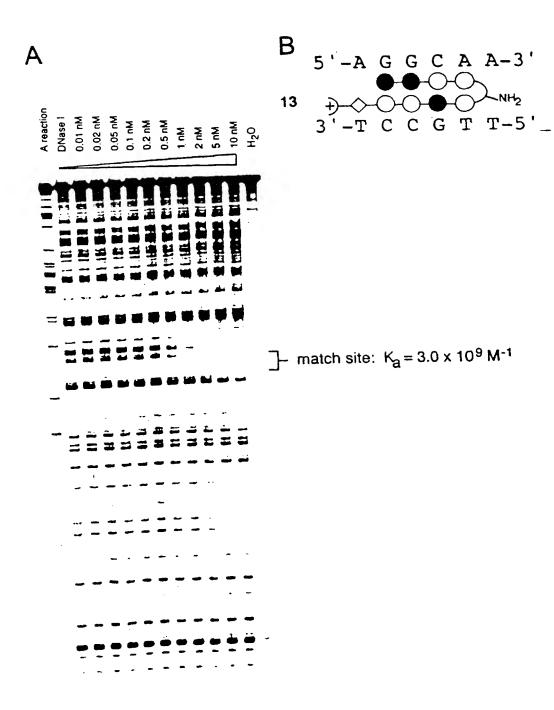
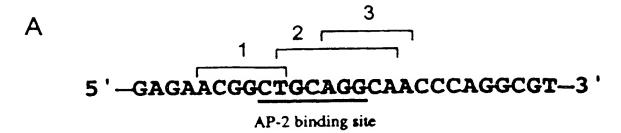


Fig. 11

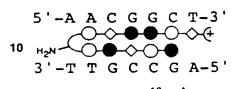
PCT/US99/20489



B

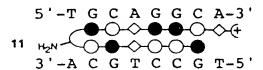
site 1

No binding observed at concentrations of 9 < 10 nM.



AACGGCT:  $K_2 = 8.7 \cdot 10^{10} \text{ M}^{-1}$  (match site) (Very selective!)

site 2



TGCAGGCA:  $K_a = 6.5 \cdot 10^9 \text{ M}^{-1}$  (match site) (Several binding sites with  $K_a = \text{ca. } 2 \cdot 10^8 \text{ M}^{-1}$ .)

TGCAGGCA:  $K_a = 5.8 \cdot 10^9 \text{ M}^{-1}$  (match site) (Several binding sites with  $K_a = \text{ca. } 2 \cdot 10^8 \text{ M}^{-1}$ .)

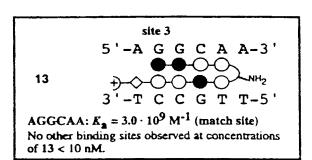


Fig. 12

PCT/US99/20489 WO 00/15773 9 ß က 2 Dist/uM ESX  $\mathbf{\omega}$ S က  $\alpha$ 

Fig. 13

PA-2E/nM

ESX

A

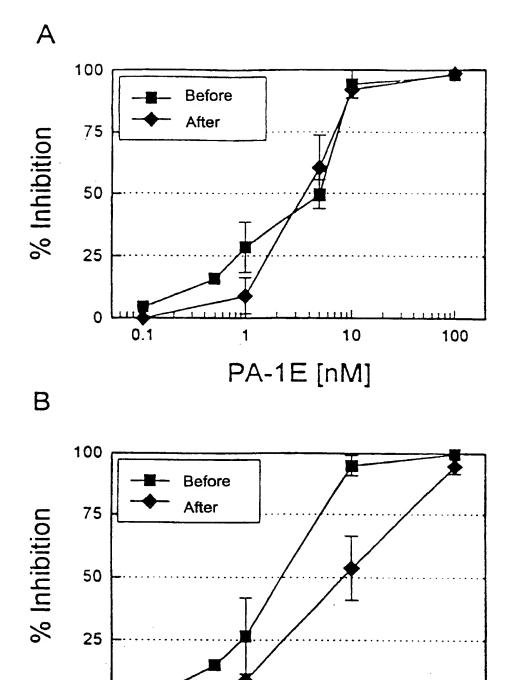


Fig. 14

PA-2E [nM]

10

100

0

0.1

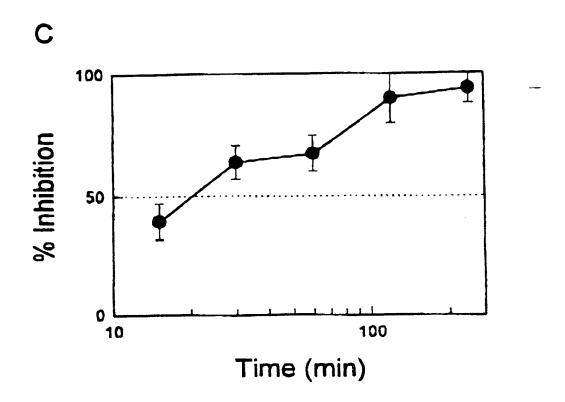
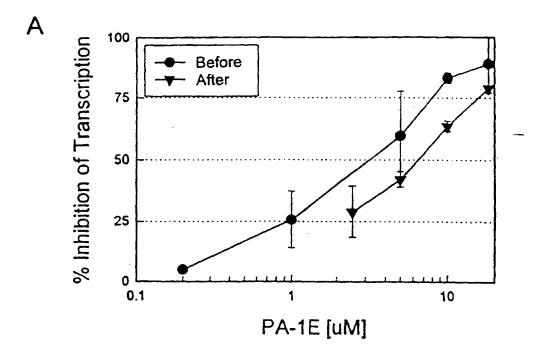
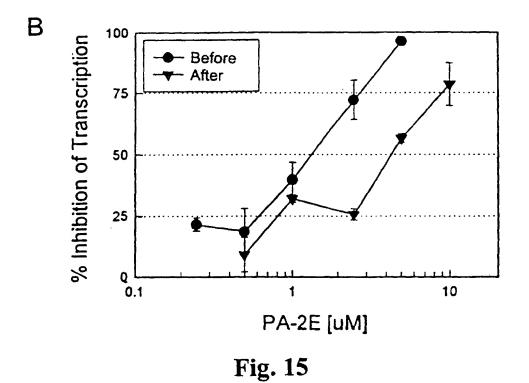


Fig. 14





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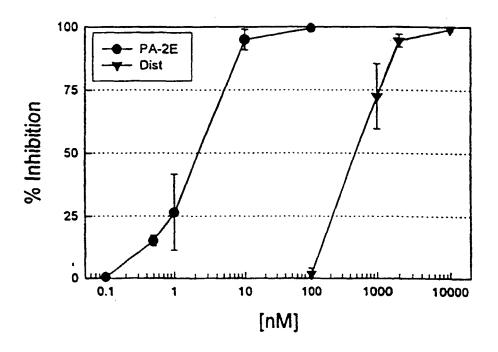


Fig. 16

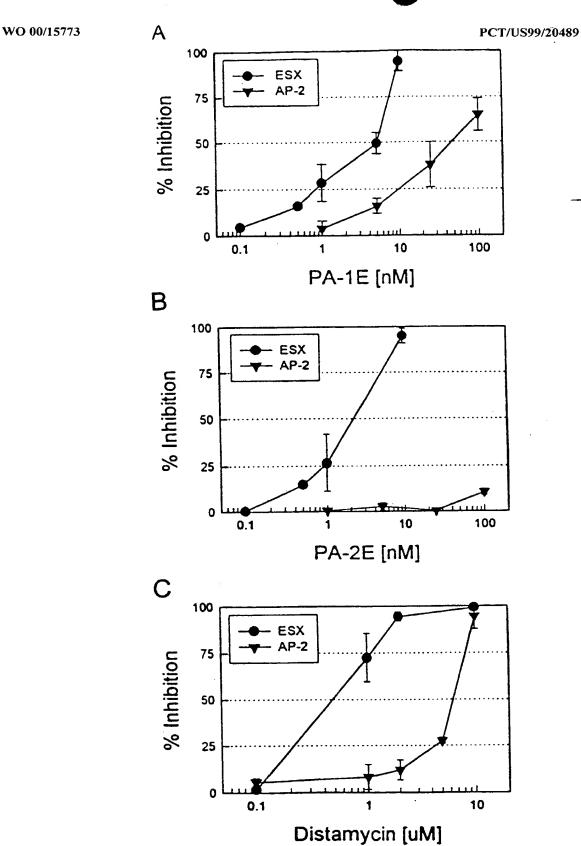


Fig. 17

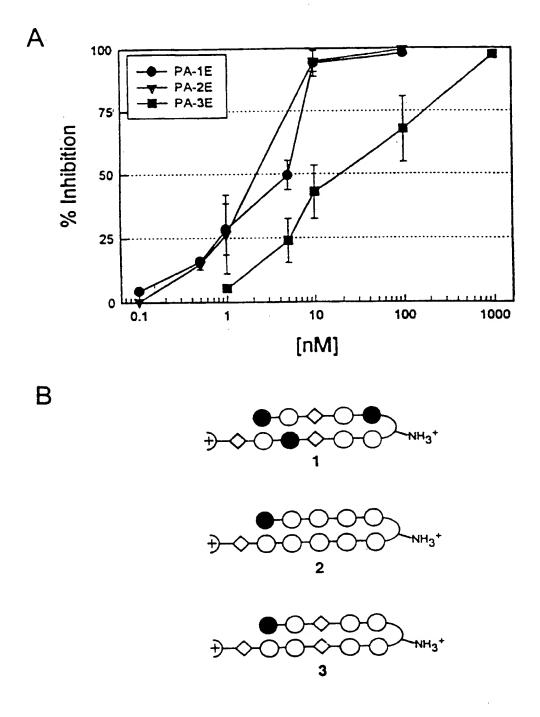


Fig. 18

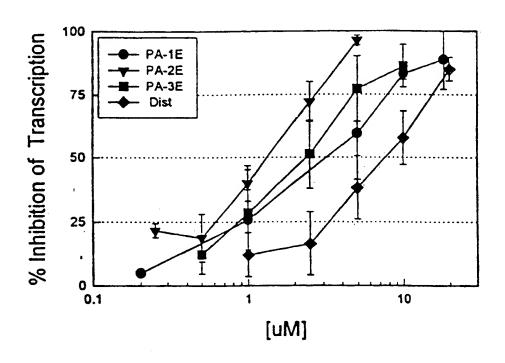


Fig. 19